INTRODUCTION AND BACKGROUND

Studies in animals have demonstrated that the cellular arm of the immune response plays the major role in eliminating murine tumors (1). Much of this evidence was derived from experiments showing that adoptive transfer of T lymphocytes from immune animals can convey resistance to a tumor challenge in recipient animals or, in some cases, results in the elimination of established cancer. Thus, most strategies for immunizing cancer patients have been directed at stimulating strong T cell immune reactions against tumor-associated antigens. Attempts at active immunization against cancer antigens have involved whole tumor cells or tumor cell fragments, however, it would be desirable to immunize specifically against unique tumor antigens that distinguish malignant from normal cells. Recently, the molecular aspect of antigen presentation to T cells and T cell antigen recognition have been defined, allowing for both discovery of defined tumor-associated antigens and better methods of immunization against the tumor-associated antigens. In contrast to antibodies that recognize epitopes on intact proteins, T cells recognize short peptide fragments (8-18 amino acids) of proteins that are processed intracellularly, then bound to and presented by the major histocompatibility complex (MHC) class I or II molecules on the cell surface (2-4).

gp100 Melanoma Antigen

Investigators in the Surgery Branch (SB), National Cancer Institute (NCI), have identified several genes that encode melanoma tumor antigens recognized by autologous or allogeneic tumor infiltrating lymphocytes (TIL) from melanoma patients in an HLA-A2 class I restricted fashion (5-8). One of these genes (gp100) was found to be widely expressed in virtually all fresh and cultured melanomas and encoded a protein identical to that recognized by the monoclonal antibody HMB-45 (9-11). Except for melanocytes and the retina, no expression of gp100 was found in normal tissues or in cancers other than melanoma. Wick et al. showed that 62 of the 67 (93%) melanomas tested (including 100% of the 62 non-spindle cell melanomas) were reactive with HMB-45, which recognizes gp100 (9). Other studies demonstrated that 32 of 35 (91%) and 60 of 62 (97%) melanomas expressed gp-100, respectively (10, 11). Four out of 14 TIL lines cultured by investigators in the SB, NCI, from different HLA-$A2^+$ melanoma patients, recognized gp100

antigens. All four of these TIL lines induced cancer regression *in vivo* after adoptive transfer into autologous patients in combination with infused recombinant interleukin-2 (6-8). Thus, gp100 antigens appear to be involved in tumor regression and may be useful in the development of immunotherapies for melanoma patients.

Vaccinia virus

Vaccinia virus is a member of the family of DNA viruses, Poxviridae, which also includes variola (smallpox virus), cowpox, monkeypox, and others (12). Unlike other DNA viruses that replicate in the cell's nucleus, these viruses replicate in the cytoplasm of the host cell. Vaccinia has been used successfully as a live vaccine for the prevention of smallpox and the eventual eradication of variola virus, the causative agent (13). Both strong humoral and cell-mediated immunity to vaccinia virus is elicited in humans exposed to this agent (14, 15). Furthermore, it is capable of carrying tumor antigens that are subsequently presented in the context of cellular MHC antigens.

Vaccinia virus has proven useful as a stable vector for replication of large foreign proteins. Recombinant vaccinia (rV) virus expression vectors are described extensively in the literature (16-18). It is ideal for recombinant research because of its relatively large genome, genetic stability, and established means of storage, transport, and delivery (15). Under the regulation of a vaccinia promoter, foreign genes are transcribed and translated, and the gene products are processed and transported in accord with the primary structure of the protein and the inherent capability of the host cell (15). Scarification with vaccinia vector results in a localized infection that allows the foreign gene to be expressed in host cells. Virus multiplies in the infected skin cells, producing greatly amplified amounts of antigen for stimulation of the immune system. Both humoral and cell-mediated immunity may be induced towards the inserted foreign gene product (19, 20).